Nutritional Evaluation of Electron Beam Irradiated *Canavalia* Seeds of Coastal Sand Dunes

Synopsis of the Ph.D. thesis
to be submitted to the

Faculty of Science, Mangalore University

in fulfilment of the requirement for the degree of

Doctor of Philosophy
in
Biosciences

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April 2011

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1. Introduction

In the developing world, food crisis is a major obstacle to humanity and can be overcome by increased food production. Plant resources having beneficial traits could be used as food. The search for novel high quality but inexpensive sources of protein and energy has continued to be a major concern in many parts of the developing world (Balogun and Fetuga, 1986). Legumes (Fabaceae) are widely used as a protein source in human diets due to high nitrogen (protein) content compared to other members of plant families. Substitution of wild plants by high yielding commercial crops is responsible for the loss of phytodiversity. Utilization of wild legumes with various functional components such as vitamins, dietary fibers and medicinal properties has focused on overcoming the nutritional deficit but the presence of antinutritional factors has limited their use as food source. The coastal sand dunes harbors wild legumes belonging to the genus *Canavalia* possessing important traits such as fast growth, mat-formation, salt tolerance and nitrogen fixation (Seena and Sridhar**,** 2006). *Canavalia* spp. also possess high quantity of carbohydrates, proteins, dietary fiber and low amounts of lipids and antinutrients (Seena and Sridhar**,** 2006; Sridhar and Seena, 2006). Antinutrients have been shown to reduce the availability of nutrients and cause toxicity if ingested in large quantities (Shim *et al.*, 2003). There are a number of food processing tools available, which serve in value addition especially in removal of antinutrients (physical, chemical and biological). One of the promising physical processing tools is food irradiation, which is a process of imparting ionizing energy to food. The ionizing energy can be in the form of gamma rays emitted from radioisotopes Cobalt-60 or Cesium-137, or X-rays or electron beams (machine-generated) sources. However, usage of gamma rays has been

hampered by unfavorable public perceptions of radioisotopes. More recently, electron beam irradiation (EBI) offer extremely rapid process of irradiation at lower cost (Cleland 1983). The food is never in contact with the energy source and the ionizing energy merely penetrates into the food but does not stay in the food. Irradiation has been recognized as a reliable and safe method for improving the nutritional value and inactivation or removal of certain antinutritional factors in foods and feeds (Siddhuraju *et al*., 2002). Irradiation has also been suggested to eliminate or reduce the antinutritional factors (Ghazy, 1990; Ghazy *et al*., 1992; Joseph and Dikshit, 1993).

The present study was conducted to elucidate some of the effects of EBI on nutritional, antinutritional and functional properties of dry seeds of the wild legume *Canavalia maritima* and *Canavalia cathartica* of the coastal sand dunes of the southwest coast of India. The impact of cooking on the nutritional, antinutritional and functional properties of the spilt beans of *C. maritima* and the effects of EBI on storage study of the spilt beans of *C. maritima* was also investigated.

2. Nutritional properties

Nutritional properties of electron beam irradiated dry seeds of *C. cathartica*, *C. maritima* and unirradiated split beans of *C. maritima* have been evaluated. Dry seeds were gathered from the dry pods of plants grown on the sand dunes of Someshwara, of the southwest coast of India (12º47′ N, 74º52′ E).The physical properties of seeds were evaluated and documented. The seeds possess distinct features especially hilum length, size and weight. The length ($P<0.001$), width ($P<0.001$), thickness ($P<0.01$) of seed and cotyledon weight ($P<0.001$) were significantly higher in *C. cathartica* than *C. maritima*. The dried seeds loaded in polypropylene bags and were exposed to different doses of electron beam irradiation (2.5 kGy, 5 kGy, 10 kGy and 15 kGy) at room temperature (25±1ºC) generated from the Microtron accelerator. Seed samples without irradiation served as control. Seed coat of the seed samples was removed and the kernels were sun-dried, milled and preserved in airtight polythene bags in refrigerator until further use.

The proximate composition of seeds of *C. maritima* did not alter at low doses but at higher doses of 10 and 15 kGy there were significant changes. A significant ($P<0.05$) decline in protein (32.11 vs. 25.98 % at 15 kGy) and fiber (1.62% vs. 0.63% at 10 kGy, 0.76% at 15 kGy) was seen but total carbohydrates showed a significant ($P<0.05$) increase at 15 kGy (60.54 vs. 65.44%). The proximate composition (fiber, oil, and calorific value) of *C. cathartica* showed significant ($P<0.05$) decrease between irradiated and non-irradiated seeds,

but in *C. maritima* the total carbohydrates (59.2 vs. 67.39% at 15kGy) showed significant increase ($P<0.05$). The ash content did not significantly alter in both seeds. However, the moisture content exhibited a dose-dependent ($P<0.05$) decrease in *C. maritima* (8.38 vs. 5.1% at 15 kGy) and *C. cathartica* (7.69 vs. 5.86%) indicating its importance in elevating shelf life due to decrease in water activity. The true proteins (24.62 vs. 14.51% at 15 kGy) and non-protein nitrogen (7.55 vs. 11.46%) were decreased significantly ($P<0.05$) at a dose of 15kGy in *C. maritima* but it was reverse in *C. cathartica*. Increase ($P<0.05$) in true proteins (16.82 vs. 23.61%) at 5 kGy and decrease ($P<0.05$) in non-protein nitrogen (12.56 vs. 4.40 %) at 5 kGy were recorded. The mineral composition of seeds of *C. maritima* and *C. cathartica* showed a slight variation. The Na/K ratio was lesser than 1 (which is favorable in prevention of high blood pressure), while the Ca/P ratio was greater than 1.0 (which prevents loss of calcium in urine) indicating the seed flours possess good qualities especially for making fabricated food. The oil extraction (FAMEs) was followed by two methods (Soxhlet method and chloroform-methanol-water method or Bligh and Dyer method). Even though seeds of two plants belonging to the same genera, they exhibited drastic change in their fatty acid profile. The amino acids were elevated and dependent on the dose of EB irradiation. Increase in essential amino acid (EAA) score, protein digestibility corrected amino acid score (PDCAAS) and protein efficiency ratio (PER) was registered in both *C. cathartica* and *C. maritima.* There was no significant change in *in vitro* protein digestibility (IVPD) of both seeds.

The antinutritional properties such as the total phenolics, canavanine and hemagglutination activity showed interesting results. Significant changes in total phenolics of *C. cathartica* (1.04 vs. 1.57% at 5 kGy was highest) and canavanine in *C. maritima* (0.031 vs. 0.035% at 10 and 15 kGy) was evident. The hemagglutination activity was not altered drastically. Hemagglutination activity of unirradiated seeds on different blood groups (O, A and B) varied between 80-140 Hu/g at lower doses (2.5 and 5 kGy), while at higher doses (10 and 15 kGy) it was 100-140 Hu/g in both seeds with a few exception.

The ripened beans of *C. maritima* were collected from ripened pods grown on the sand dunes of Someshwara. After removal of testa and seed coat, the cotyledons were split, sun-dried, powdered and preserved in airtight glass containers. The second portion of the sample (split beans) was cooked in a pressure cooker. The cooked samples were sun-dried drained and powdered. The physical features of the ripened spilt beans were evaluated.

The proximate composition of split beans showed a significant decrease ($P<0.001$) in the moisture (8.11 vs. 6.09) and oil content (3.27 vs. 2.68 %) but an elevation ($P<0.01$) was

seen in fibre (0.76 vs. 1.28%), carbohydrates and calorific value. There were also no alterations in protein and ash content. The mineral composition was similar to that of other legumes containing K, Mg, Fe, Zn and Ca. The Na (36.73 vs. 44.83 mg/100 g), P (149.27 vs. 154.27 mg/100 g), Fe (12.56 vs. 13.80 mg/100 g) and Zn (2.32 vs. 2.36 mg/100 g) increased significantly ($P<0.01$) on cooking. However, the Se was also found in split beans, which serve as antioxidant in small quantities. As dry seeds, the Na/K ratio was lesser than 1, while the Ca/P ratio was greater than 1.0 in split beans shows its potency to serve as health food. The Bligh and Dyer method showed lower saturated fatty acids than soxhlet method. A strong milky aroma was seen in split beans (due to high myristic acid). The oleic acid was highest among the unsaturated fatty acids by soxhlet extraction and has wide application in aerosol products as an emulsifier. The ω-6 to ω-3 fatty acid ratio of split bean was 5.14 which fulfils the FAO-WHO recommended range (5-10).

In split beans, protein fractions include albumin, globulin, prolamin and glutelin. The water soluble protein fractions namely albumin and globulin were predominant than reserve proteins such as prolamin and glutelin in ripened kernels of *C. maritima*. The albumin, prolamin and glutelin increased but the globulin decreased (7.66 vs. 1.56%, $P<0.01$) drastically on cooking. Increase in albumin and decrease in globulin indicates higher nutritive value of the cooked split bean flour. Proteolysis of prolamin increases the peptides rich in glutamine. The NPN content increased on cooking. Protein fractions serve as an indicator in assessing protein quality of food. The essential amino acids increased on cooking with elevation in PDCAAS as well as PER. The IVPD of the uncooked pulse (61.91%) was higher than that of *Vicia faba* (53%) and *Vigna mungo* (56%). On cooking the IVPD showed a significantly increase (68.24% vs. 61.91%) to that of uncooked split beans. The protein digestibility of the cooked split beans was higher than *Vigna radiata* (67%). Improvement in the protein digestibility after cooking may be partly due to reduction in the levels of various antinutrients such as total phenolics (1.7 vs. 1.13 %, $P<0.05$), canavanine (0.03 vs. 0.02%, $P<0.001$), hemagglutination and also due to the increased accessibility of the proteins for enzymatic attack.

3. Functional properties

The functional properties (water absorption, oil absorption, foam capacity, foam stability, emulsion activity, emulsion stability and least gelation capacity) of the electron beam irradiated dry seeds of *Canavalia* spp. were assessed. Difference was found only in gelation and foam capacity. The least gelation capacity of unirradiated seed flour of *C.*

maritima was at 8%, while for all the doses of irradiation it was at 6%. In *C. cathartica*, it was at 8% except for the flours of 5 kGy. The foam capacity of *C. maritima* between unirradiated and selected doses of irradiation (5 kGy and 10 kGy; $P<0.05$) was significantly differed. The functional properties of the raw and cooked spilt beans were also studied. Water absorption capacity of the uncooked spilt beans was higher than the cooked split beans, which may be due to the water retention ability of the flour. The gelation capacity of the spilt bean was 8% in uncooked and 6% in cooked spilt beans. The ability of protein to form gels provides a structural matrix for holding water, flavors and sugars, which is useful in development of new food stuffs. The low least gelation capacity of both uncooked and cooked flours of split beans indicates their greater ability to form gels than other seed flours. The foam capacity of uncooked split beans (18%) was higher than cooked beans (6%). The foam stability of the uncooked split bean flour was 40.74% but the cooked spilt bean failed to retain the stability. The oil absorption capacity, emulsion activity and emulsion stability although decreased on cooking, they were not significant ($P>0.05$).

3. Fatty acid profile

The EBI significantly increased total saturated fatty acids of *C. maritima* seeds, increase in total saturated fatty acids (11.3 vs. 22.4–30.5 g/kg) was significant at 2.5 ($P<0.01$), 5 and 15 kGy ($P<0.05$) in the soxhlet method, but there was no significant increase on extraction by the Bligh and Dyer method. In *C. cathartica* seeds exposed to 2.5–10 kGy on extraction by soxhlet (15.8 vs. 22.2–26.2 g/kg; $P<0.05$) as well as Bligh and Dyer (5.04 vs. 9.8–10.9 g/kg; $P<0.05$) methods there was a significant increase. In *C. maritima* seeds, the total unsaturated fatty acids significantly decreased in the soxhlet method up to 10 kGy (14.3 vs. 3.6–10.2 g/kg; $P<0.05$), while it was significantly elevated at 15 kGy (17.6 g/kg; $P<0.05$). But, extraction by the Bligh and Dyer method showed significant increase in the total unsaturated fatty acids in irradiated seeds of *C. maritima* at 2.5 (13.9 vs. 20.6; $P<0.01$) and 5 kGy (13.9 vs. 22.9 g/kg; $P<0.05$). The total unsaturated fatty acids was significantly decreased in *C. cathartica* seeds (39.8 vs. 4.9–10.9 g/kg; $P<0.01$) in all EBI doses on extraction by the soxhlet method. In the Bligh and Dyer method, it significantly elevated at 5 and 10 kGy (17.04 vs. 29.4–31.4 g/kg; $P<0.05$). The seeds of *C. cathartica* were endowed with almost threefold higher total unsaturated fatty acids than *C. maritima* as revealed by the soxhlet method (14.3 vs. 39.8 g/kg). Similarly, the Bligh and Dyer method also revealed higher total unsaturated fatty acids in *C. cathartica* seeds than *C. maritima* (13.9 vs. 17.04 g/kg). It is likely that the Bligh and Dyer method showed a significant increase of the total

unsaturated fatty acids in irradiated *Canavalia* seeds as this method is devoid of temperature treatment unlike the soxhlet method.

The myristic acid by soxhlet extraction showed a significant elevation in irradiated seeds .The flour of irradiated *Canavalia* seeds possessed a strong milky aroma possibly due to high quantity of myristic acid. Medium chain fatty acids (capric and caprylic) increased on irradiation. Among the unsaturated fatty acids, oleic acid decreased (*C. maritima*, 10.6 vs. 0.1-7.3 g/kg; *C. cathartica*, 2-3.8 vs. 3.4-7.4 g/kg) on irradiation (Soxhlet method) but showed an increase in Bligh and Dyer methods. Linoleic acid showed an increase which attained the highest at 5 (*C. maritime*: 2.38 vs. 3.74 g/kg) and 10 kGy (*C. cathartica*: 3.3 vs. 5.44 g/kg) in Bligh and Dyer method. The concentration of linolenic acid significantly elevated on irradiation reaching the highest at 5 kGy both in *C. maritima* (1.3 vs. 2 g/kg) and *C. cathartica* (1.5 vs. 2.5 g/kg) extracted by Bligh and Dyer methods. The study revealed that in both control and irradiated *Canavalia* seeds, the ω-6/ω-3 ratio is lower than FAO-WHO (1998) recommended pattern (1.4-4.7 vs. 5-10) indicating more balanced selective ratios can be achieved through EBI.

5. Storage study

The above study registered that the spilt beans are nutritionally an excellent source. However, they seem to be susceptible for fungal and insect infestation. The shelf life of the split beans was elevated by exposure to EBI (2.5, 5, 10 and 15 kGy). The irradiated spilt beans were stored for different intervals (0, 3 and 6 months). The dominance of fungi depends upon the existing environmental conditions. A drastic decline in the number of fungal species as well as fungal incidence was seen in irradiated samples. The EBI showed a dose dependent decline in percent incidence up to 5 kGy but complete decontamination occurred at 10 and 15 kGy. The control samples were prone to contamination and decoloration after 6 months preservation, but the samples exposed to higher doses (10 and 15 kGy) of irradiation were devoid of contamination as well as discoloration.

6. Outlook

The crude protein content of the *C. maritima* (26 g/100 g) showed a significant decrease at 15 kgy but was higher than *Vigna radiata* (18.5 g/100 g) and *V. unguiculata* (14 g/100 g). Protein elevation was not significant due to the complex matrix of food stuff, which was resistant to radiation treatment. Oil content of *C. cathartica* on irradiation showed a significant decrease (5.9% vs. 3.01%) but the unirradiated samples showed higher than *Vigna*

radiata (5.3%) and *V. unguiculata* (3.4%). Significant decrease in crude fiber on irradiation at higher doses may be attributed to depolymerisation and delignification. The total carbohydrates showed a dose-dependent elevation. Food source is considered good if the Na/K ratio <1 and Ca/P ratio is >1. The present investigations have proved that dry seeds of *Canavalia* spp. possess desired Na/K and Ca/P ratios. Among the methods employed, Bligh and Dyer method served the best and the low doses (2.5 and 5 kGy) serves as a hormetic dose to preserve and selectively change the fatty acid composition of dry seeds. Increase in EAA reflected in the EAA score, PDCAAS and PER. The PER is helpful in evaluating the protein quality. If the PER is <1 the food is poor in protein, if it ranges from 1.5-2 it is moderate in nutritional qualities and if it is >2 it is good in protein. The current study revealed higher PER (>2) in *Canavalia* seeds and thus serve as good protein source.

Although an increase in total phenolics of irradiated *C. cathartica* (1.57%) was seen it was lower than *C. gladiata* (1.82%) comparable to *Vicia faba* and higher than some cultivated legumes. The adverse effects arise from the presence of some of the antinutritional factors in the raw seeds including L-canavanine. Seeds of *Canavalia* spp. showed the presence of canavanine in the range of 0.023-0.031%, which was reported to be lower than the lethal concentration (0.035-0.11%). Moreover, the canavanine at the dose in *Canavalia* seeds seems to combat the colon cancer. The EBI brought partially favorable changes in *Canavalia* seed nutritional and functional properties without affecting the protein qualities. Pressure cooking was more favorable than irradiation by lowering the antinutritional components in split beans. However, the EBI of split beans helped in elevation of shelf life. The adaptability and nutritional advantages of seeds of *Canavalia* spp. (e.g. raw, germinated, ripened and tender seeds) indicate the possibility to develop new viable crops for human consumption with nutraceutical value.

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